

SEQUENCE LISTING

<110> ZHANG, HUANMIN

AX, ROY L

BELLIN, MARY E

<120> ISOLATED POLYNUCLEOTIDE SEQUENCES ENCODING A FERTILITY ASSOCIATED ANTIGEN

05051407101  
460  
30> 210707US20

40> US 60/218,140

41> 2000-07-14

42> 9

<170> PatentIn version 3.1

<210> 1

<211> 592

<212> DNA

<213> Bos sp.

<220>

<221> CDS

<222> (1)..(591)

<223>

<400> 1																			
gag aag cta aac gga aat tca aga aaa ggc ata aca tac aac tat gtg																			48
Glu Lys Leu Asn Gly Asn Ser Arg Lys Gly Ile Thr Tyr Asn Tyr Val																			
1 5 10 15																			
att agc tct cgc ctt gga aga aac aca tat aaa gaa cag tat gcc ttt																			96
Ile Ser Ser Arg Leu Gly Arg Asn Thr Tyr Lys Glu Gln Tyr Ala Phe																			
20 25 30																			
ctc tat aaa gaa aag cta gtg tct gta aaa caa agc tac ctc tac cac																			144
Leu Tyr Lys Glu Lys Leu Val Ser Val Lys Gln Ser Tyr Leu Tyr His																			
35 40 45																			
gac tat cag gct gga gac gca gat gtg ttt tcc agg gaa ccc ttt gtg																			192
Asp Tyr Gln Ala Gly Asp Ala Asp Val Phe Ser Arg Glu Pro Phe Val																			
50 55 60																			
ctc tgg ttc cag tca ccc tac acc gct gtc aag gac ttc gtg att gtc																			240
Val Trp Phe Gln Ser Pro Tyr Thr Ala Val Lys Asp Phe Val Ile Val																			
70 75 80																			
ccc ctg cac acc acc cct gag aca tcc gtt aga gag att gat gag ctg																			288
Pro Leu His Thr Thr Pro Glu Thr Ser Val Arg Glu Ile Asp Glu Leu																			
85 90 95																			
gct gat gtc tac aca gat gtg aaa cgt cgc tgg aat gca gag aat ttc																			336
Ala Asp Val Tyr Thr Asp Val Lys Arg Arg Trp Asn Ala Glu Asn Phe																			
100 105 110																			
att ttc atg ggt gac ttc aat gct ggc tgc agc tac gtc ccc aag aag																			384
Ile Phe Met Gly Asp Phe Asn Ala Gly Cys Ser Tyr Val Pro Lys Lys																			
115 120 125																			
gcc tgg aag gac atc cgc ctg agg acg gac ccc aag ttc gtt tgg ctg																			432
Ala Trp Lys Asp Ile Arg Leu Arg Thr Asp Pro Lys Phe Val Trp Leu																			
130 135 140																			
atc ggg gac caa gag gac acc acg gtc aag aag agc aca aac tgc gcc																			480
Ile Gly Asp Gln Glu Asp Thr Thr Val Lys Lys Ser Thr Asn Cys Ala																			
145 150 155 160																			
tat gac agg atc gtg ctt aga gga caa aat att gtc aac tct ggt ggt																			528
Tyr Asp Arg Ile Val Leu Arg Gly Gln Asn Ile Val Asn Ser Gly Gly																			
165 170 175																			
cct caa tca aac ctc gtc ttt gat ttc cag aaa gct tac agg ttg tct																			576
Pro Gln Ser Asn Leu Val Phe Asp Phe Gln Lys Ala Tyr Arg Leu Ser																			
180 185 190																			
gaa tcg aag gcc ctg g																			592



Ile Gly Asp Gln Glu Asp Thr Thr Val Lys Lys Ser Thr Asn Cys Ala  
 145 150 155 160

Tyr Asp Arg Ile Val Leu Arg Gly Gln Asn Ile Val Asn Ser Gly Gly  
 165 170 175

Pro Gln Ser Asn Leu Val Phe Asp Phe Gln Lys Ala Tyr Arg Leu Ser  
 180 185 190

Glu Ser Lys Ala Leu  
 195

<210> 3

<211> 671

<212> DNA

<213> Bos sp.

<240> 3

acacaggat ctgccccata ctgatggaga agctaaacgg aaattcaaga aaaggcataa 60  
 catacaacta tgtgattagc tctcgccttg gaagaaacac atataaagaa cagtatgcct 120  
 ttctctataa agaaaagcta gtgtctgtaa aacaaagcta cctctaccac gactatcagg 180  
 ctggagacgc agatgtgttt tccagggaac cctttgtggt ctggttccag tcaccctaca 240  
 ccgctgtcaa ggacttcgtg attgtcccc tgcacaccac ccctgagaca tccgtagag 300  
 agattgatga gctggctgat gtctacacag atgtgaaacg tcgctggaat gcagagaatt 360  
 tcattttcat gggtgacttc aatgctggct gcagctacgt cccaagaag gcctggaagg 420  
 acatccgcct gaggacggac cccaagtctg tttggctgat cggggaccaa gaggacacca 480  
 cgggtcaagaa gagcacaac tgcgcctatg acaggatcgt gcttagagga caaaatattg 540  
 tcaactctgg tggctctcaa tcaaacctcg tctttgattt ccagaaagct tacaggttgt 600  
 ctgaatcgaa ggccttgat gtcagcgacc actttccagt tcatcatcat catcatcatg 660  
 aagaaccatg a 671

<210> 4  
 <211> 21  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> synthetic DNA

<400> 4  
 cgtgaggagc ttcggcgaga g

21

<210> 5  
 <211> 26  
 <212> PRT  
 <213> Bos sp.

<220>  
 <221> misc\_feature  
 <222> (4)..(4)  
 <223> Xaa is any amino acid

<400> 5  
 Leu Lys Ile Xaa Ser Phe Asn Val Arg Ser Phe Gly Glu Ser Lys Lys  
 1 5 10 15

Ala Gly Phe Asn Ala Met Arg Val Ile Val  
 20 25

<210> 6  
 <211> 26

<212> DNA

<213> Artificial Sequence

<220>

<223> synthetic DNA

<400> 6  
acaacaggat ctgccccata ctgatg

26

<210> 7

<211> 25

<212> DNA

<213> Artificial Sequence

<220>

<223> synthetic DNA

<400> 7  
caactggaa agtggtcgct gacat

25

<210> 8

<211> 27

<212> DNA

<213> Artificial Sequence

<220>

<223> synthetic DNA

<400> 8  
acaacaggat ctgccccata ctgatgg

27

<210> 9

<211> 57

<212> DNA

<213> Artificial Sequence

<220>

<223> synthetic DNA

<400> 9

tcatgggttct tcatgatgat gatgatgatg aactggaaag tggtcgctga catccag

57

0990514.071501



Figure 1. Depicted recombinant FAA (rFAA), produced from cloned partial cDNA of bovine FAA gene in *E. coli*, showing the comparative position of the segment corresponding to intact bovine FAA.



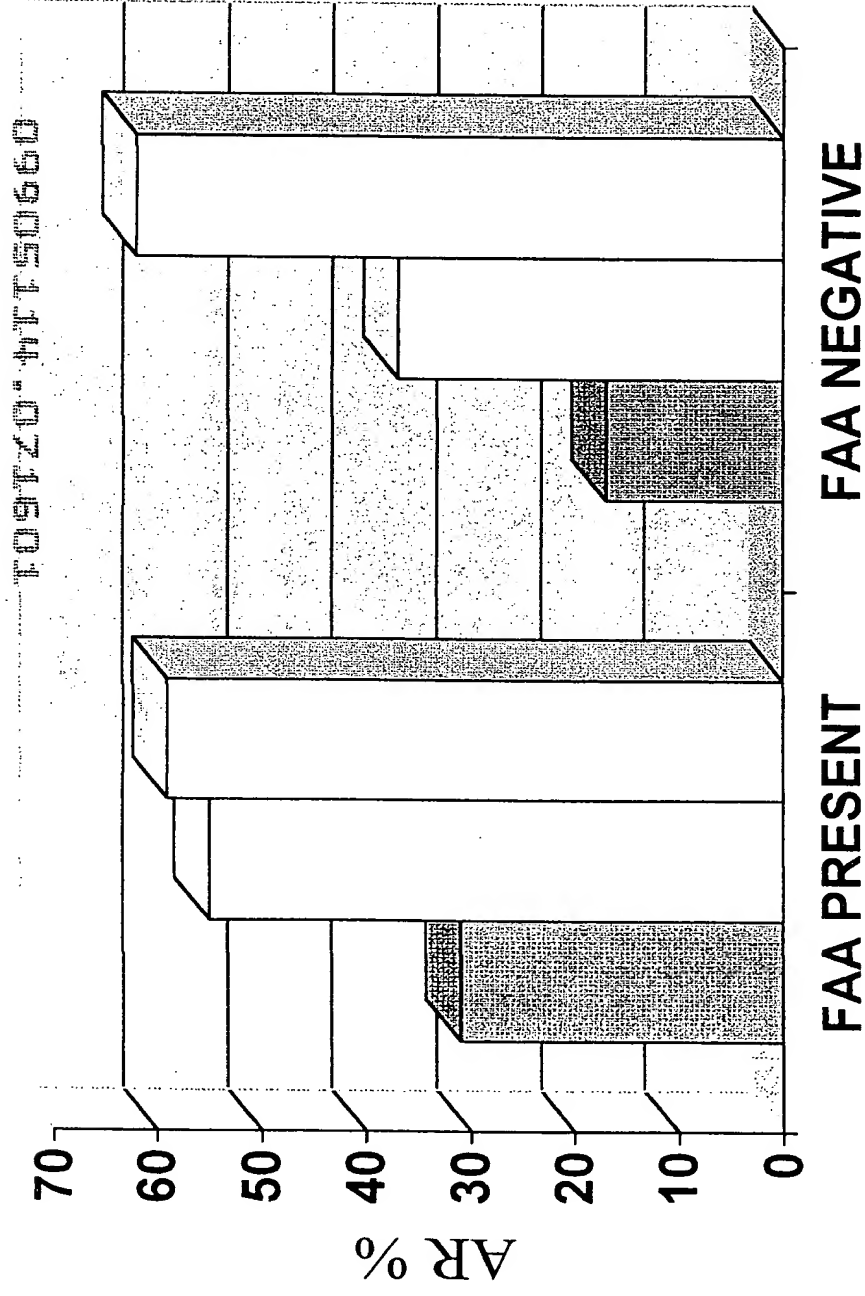


Figure 2. Percent increase in acrosome reaction for each treatment above the control level. FAA present represents a fertile bull with detectable FAA on sperm and FAA negative represents a non-fertile bull without detectable FAA on sperm. The fertile bull (FAA present) reacted better to heparin induction of capacitation/acrosome reactions. Addition of FAA (5 ug/mL) stimulated maximum increase of acrosome reactions for each bull.

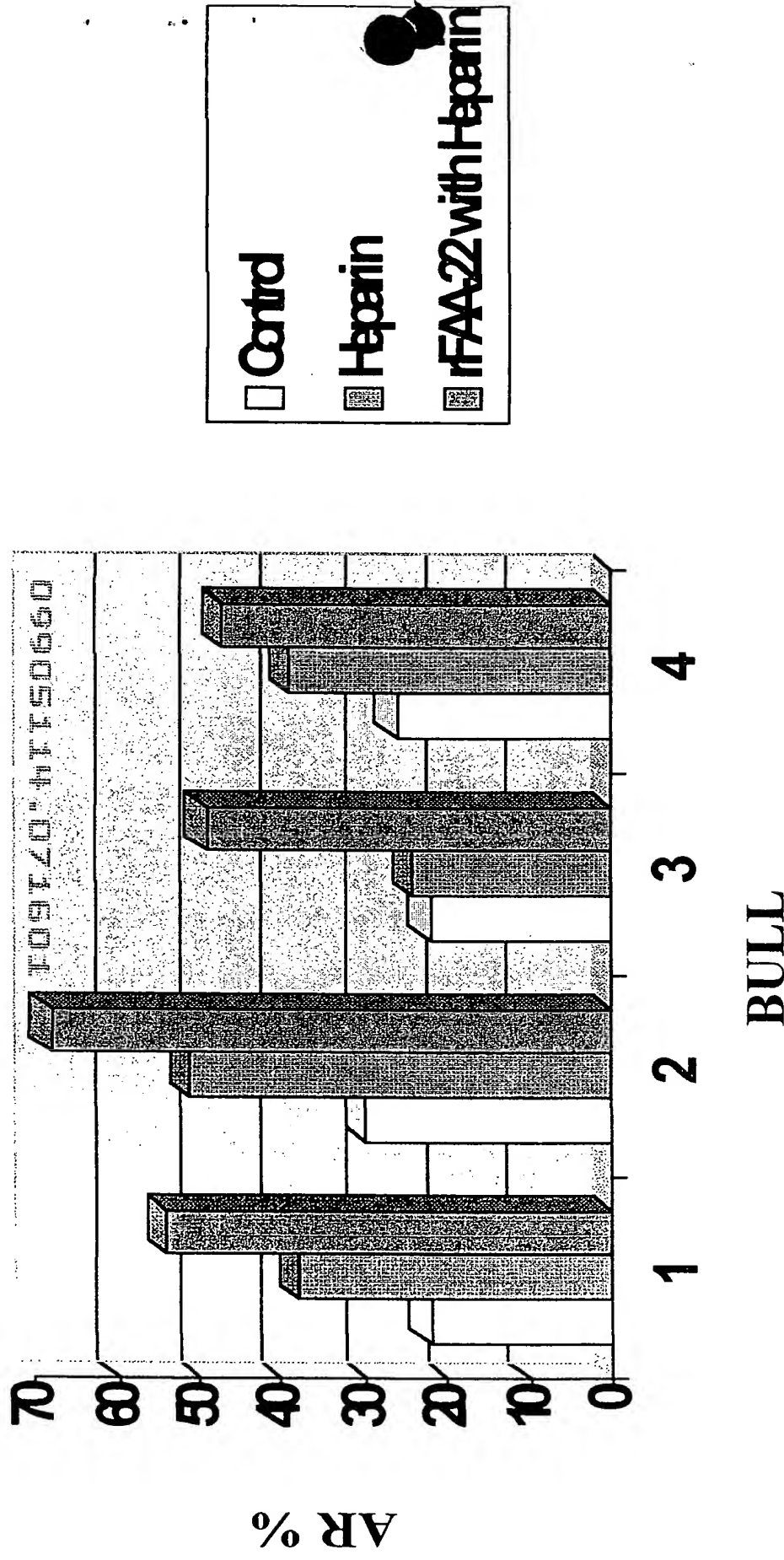


Figure 3. Effects of heparin alone (10  $\mu\text{g}/\text{ml}$ ) or recombinant FAA (rFAA, 20  $\mu\text{g}/\text{ml}$ ) with heparin to stimulate acrosome reaction in washed sperm from four fertile bulls.

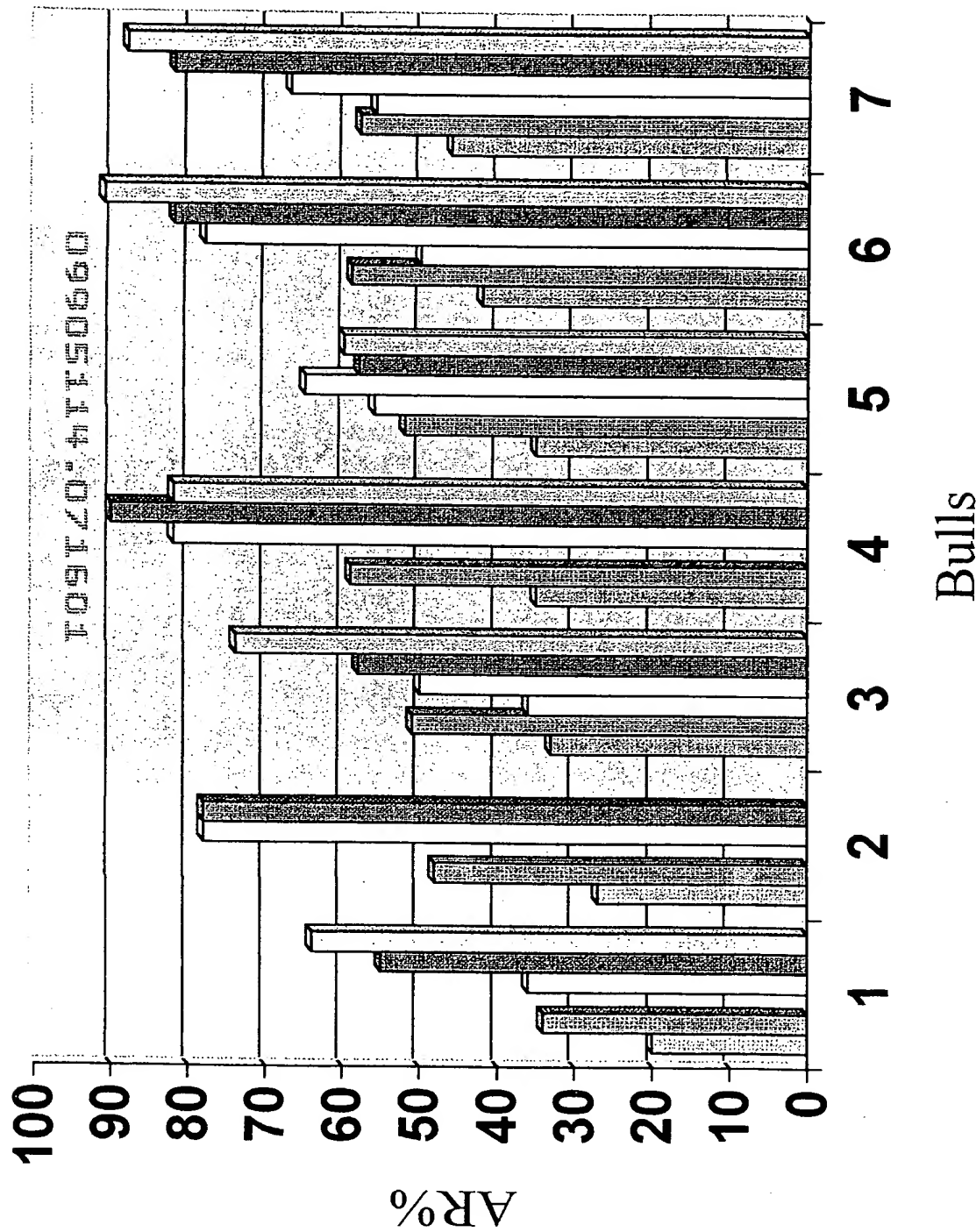


Figure 4. Dose-response comparisons ( $\mu\text{g/ml}$ ) of the 22kDa recombinant FAA (rFAA) added with heparin ( $10 \mu\text{g/ml}$ ) to washed sperm. Averages from two to five different ejaculates are presented as each datum point for each bull.

Thursday, July 06, 2000

1 ACAACAGGAT CTGCCCCATA CTGATGGAGA AGCTAAACGG AAATTCAAGA  
51 AAAGGCATAA CATACAACTA TGTGATTAGC TCTCGCCTTG GAAGAAACAC  
101 ATATAAAGAA CAGTATGCCT TTCTCTATAA AGAAAAGCTA GTGTCTGTAA  
151 AACAAAGCTA CCTCTACCAC GACTATCAGG CTGGAGACGC AGATGTGTTT  
201 TCCAGGGAAC CCTTTGTGGT CTGGTTCCAG TCACCCTACA CCGCTGTCAA  
251 GGACTTCGTG ATTGTCCCCC TGCACACCAC CCCTGAGACA TCCGTTAGAG  
301 AGATTGATGA GCTGGCTGAT GTCTACACAG ATGTGAAACG TCGCTGGAAT  
351 GCAGAGAATT TCATTTTTCAT GGGTGACTTC AATGCTGGCT GCAGCTACGT  
401 CCCCAAGAAG GCCTGGAAGG ACATCCGCCT GAGGACGGAC CCCAAGTTCG  
451 TTTGGCTGAT CGGGGACCAA GAGGACACCA CGGTCAAGAA GAGCACAAAC  
501 TGCGCCTATG ACAGGATCGT GCTTAGAGGA CAAAATATTG TCAACTCTGG  
551 TGGTCCTCAA TCAAACCTCG TCTTTGATTT CCAGAAAGCT TACAGGTTGT  
601 CTGAATCGAA GGCCCTGGAT GTCAGCGACC ACTTTCCAGT TCATCATCAT  
651 CATCATCATG AAGAACCATG A

Notes: Upstream primer sequence;

**Codon sequence responsible for the rFAA product;**

Stop codon.

090514.071601

Figure 6

5' GAGAAGCTAAACGGAAATTCAAGAAAAGGCATAACATACAACTATGTGATTAGCTCTCGC  
1 -----+-----+-----+-----+-----+-----+ 60  
a E K L N G N S R K G I T Y N Y V I S S R -  
  
CTTGGAAGAAACACATATAAAGAACAGTATGCCTTTCTCTATAAAGAAAAGCTAGTGTCT  
61 -----+-----+-----+-----+-----+-----+ 120  
a L G R N T Y K E Q Y A F L Y K E K L V S -  
  
GTAAAACAAAGCTACCTCTACCACGACTATCAGGCTGGAGACGCAGATGTGTTTTCCAGG  
121 -----+-----+-----+-----+-----+-----+ 180  
V K Q S Y L Y H D Y Q A G D A D V F S R -  
  
GAACCCTTTGTGGTCTGGTTCCAGTCACCCTACACCGCTGTCAAGGACTTCGTGATTGTC  
181 -----+-----+-----+-----+-----+-----+ 240  
E P F V V W F Q S P Y T A V K D F V I V -  
  
CCCCTGCACACCACCCTGAGACATCCGTTAGAGAGATTGATGAGCTGGCTGATGTCTAC  
241 -----+-----+-----+-----+-----+-----+ 300  
P L H T T P E T S V R E I D E L A D V Y -  
  
ACAGATGTGAAACGTCGCTGGAATGCAGAGAATTTTCATTTTCATGGGTGACTTCAATGCT  
301 -----+-----+-----+-----+-----+-----+ 360  
T D V K R R W N A E N F I F M G D F N A -  
  
GGCTGCAGCTACGTCCCCAAGAAGGCCTGGAAGGACATCCGCCTGAGGACGGACCCCAAG  
361 -----+-----+-----+-----+-----+-----+ 420  
a G C S Y V P K K A W K D I R L R T D P K -  
  
TTCGTTTGGCTGATCGGGGACCAAGAGGACACCACGGTCAAGAAGAGCACAAACTGCGCC  
421 -----+-----+-----+-----+-----+-----+ 480  
a F V W L I G D Q E D T T V K K S T N C A -  
  
TATGACAGGATCGTGCTTAGAGGACAAAATATTGTCAACTCTGGTGGTCCTCAATCAAAC  
481 -----+-----+-----+-----+-----+-----+ 540  
a Y D R I V L R G Q N I V N S G G P Q S N -  
  
CTCGTCTTTGATTTCAGAAAGCTTACAGGTTGTCTGAATCGAAGGCCCTGG 3'  
541 -----+-----+-----+-----+-----+-----+ 592  
a L V F D F Q K A Y R L S E S K A L -

1091204420560